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Detection of Spliceomic Signatures for Predicting Endocrine Resistance in Estrogen Receptor-positive Breast Cancer

***Han-Byoel Lee*[1]**, Min-Su Kim[2], Jiyoung Rhu, MD[1], Jung Hyun Park[1], Kyung Eun Kim[1], Young Wook Ju[1], Eun-Shin Lee[1], Hyeong-Gon Moon, MD, PhD[1,3], Dong-Young Noh, MD, PhD[1,3], Sun Kim, PhD[4], Wonshik Han, MD, PhD[1,3]

[1]Dept. of Surgery, Seoul National University Hospital, Seoul National University College of Medicine
[2]Interdisciplinary Program in Bioinformatics, [3]Cancer Research Institute, [4]Department of Computer Science and Engineering, Interdisciplinary Program in Bioinformatics, and Bioinformatics Institute, Seoul National University

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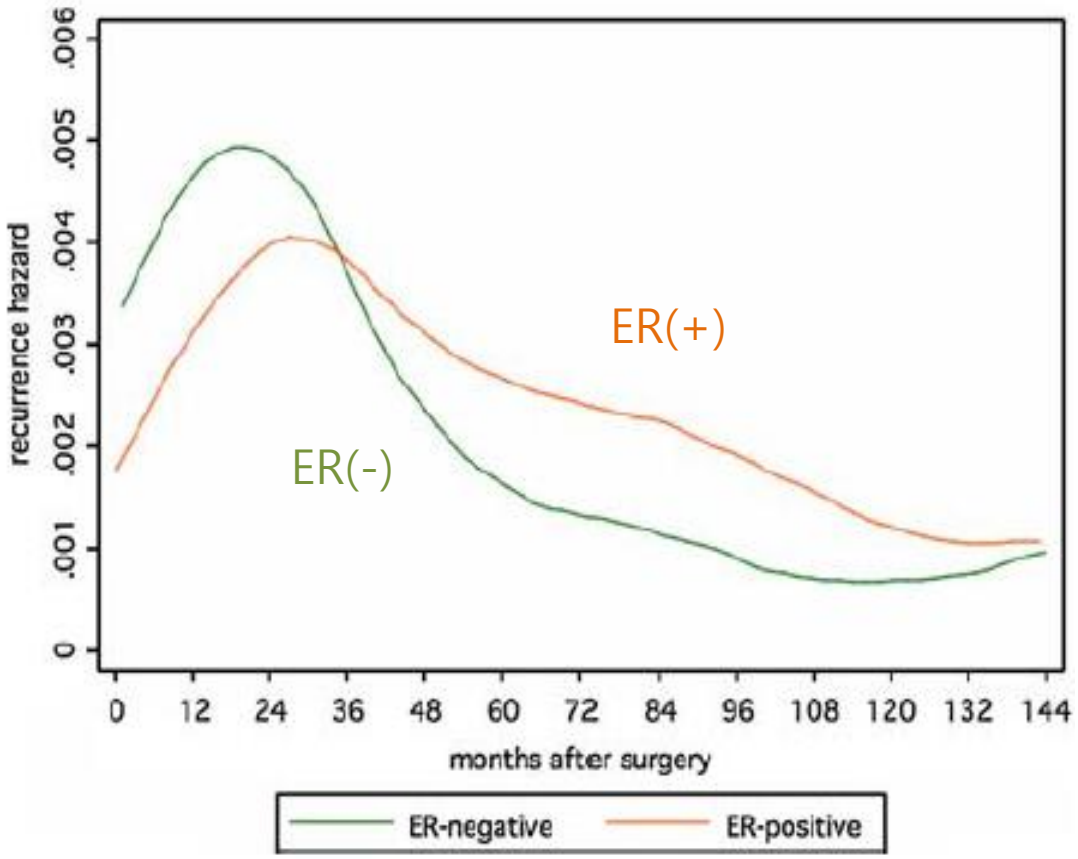
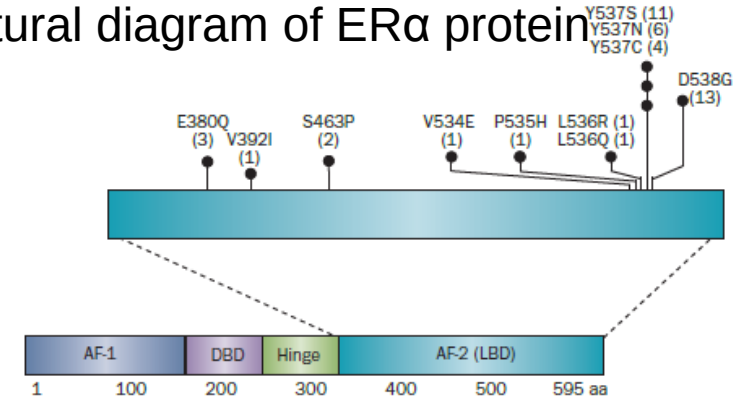
Introduction

- ✓ 70% of breast cancers are HR(+)
- ✓ Relatively indolent subtype
- ✓ Endocrine resistance in 25%



Structural diagram of ERα protein

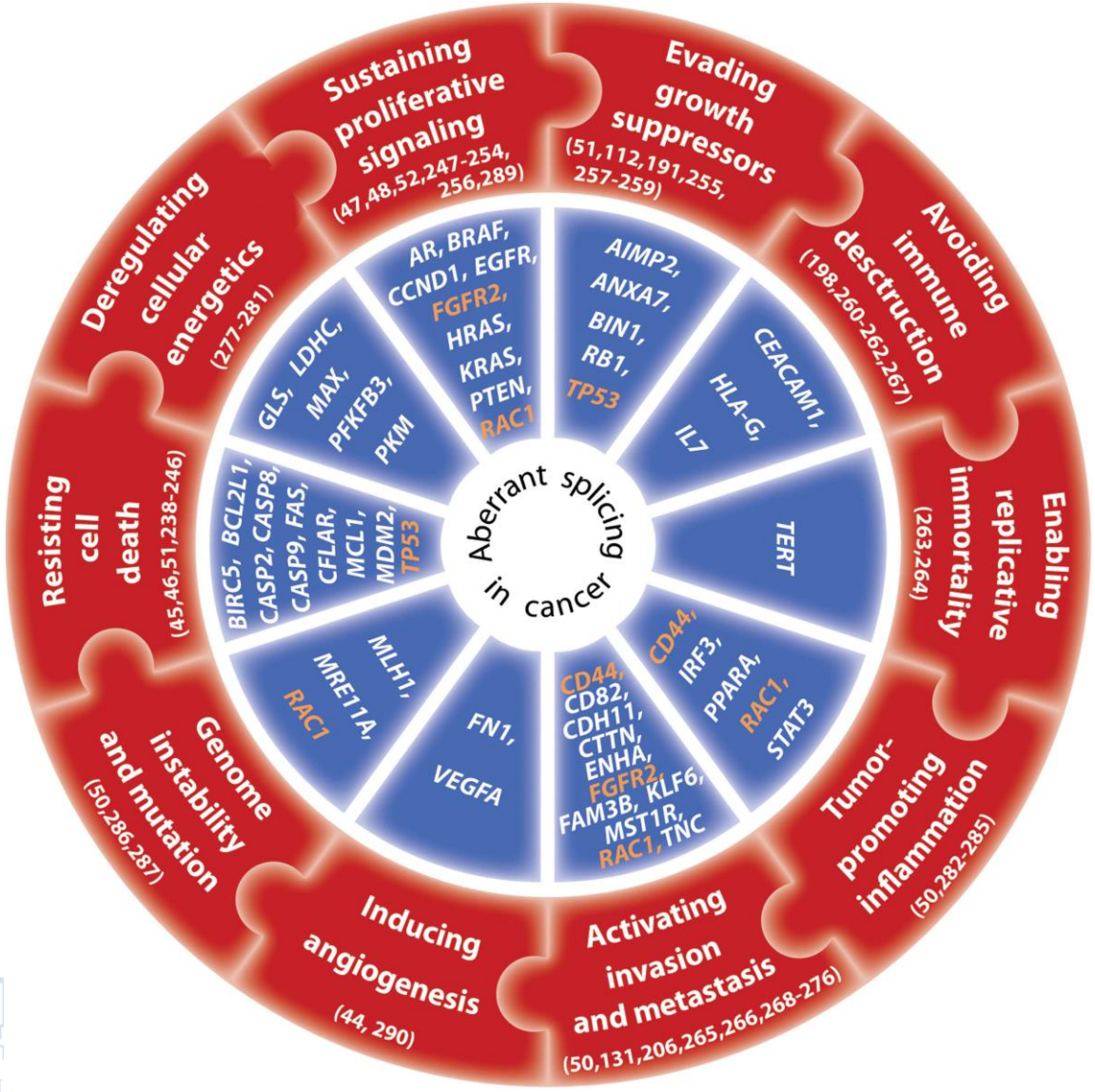


- ✓ *ESR1* mutations found in 20% of metastatic ER(+) breast cancer
- ✓ Vast majority of the alterations are missense mutations within the ER ligand binding domain

- ✓ cf. *ESR1* mutation in 0.5% of primary tumor (TCGA)
- ✓ Only 8 genes were mutated in more than 5%
  - ✓ *PIK3CA, TP53, MAP3K1, MAP2K4, GATA3, MLL3, CDH1, PTEN*

Dignam et al. Breast Cancer res Treat 2009. PMID: 18830816
Jeselsohn et al. Nat Rev Clin Oncol 2015. PMID: 26122181

Alternative Splicing



- ✓ There are more than 10-thousands isoforms expressed from 30-thousands genes in human tissues

Pan et al., Nat Genet 2008.

- ✓ AS is also a tightly regulated process, which is systematically controlled and even inherited

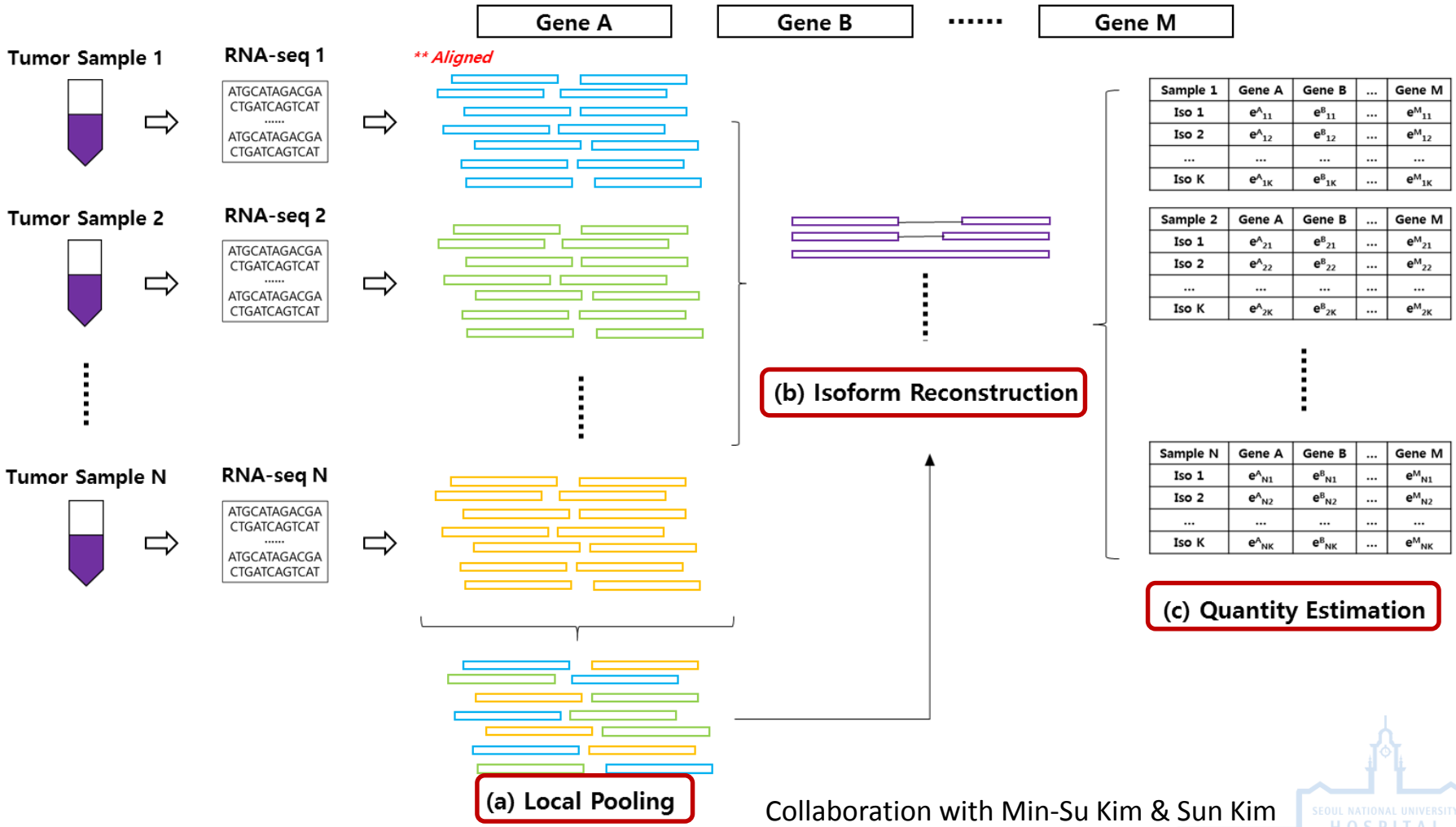
Chen, et al., Cancer Discov 2015.

- ✓ Aberrant splicing events are observed in almost every hallmarks of cancer, implying that mis-regulation of splicing and cancer progression are closely related.

Sveen et al., Oncogene 2015.

Cancer Spliceome Pre-processing Pipeline (SpliceHetero)

- ✓ A pipeline designed to accurately produce ***spliceomic profiles*** (cf. spliceosome)
- ✓ from large cohorts of tumor RNA-sequencing data,
- ✓ taking into account ***tumor heterogeneity.***



Collaboration with Min-Su Kim & Sun Kim

Purpose

✓The purpose of this study was

to detect phenotype-specific splice variants,

and to discover **spliceomic signatures** related to

endocrine resistance in HR-positive breast cancer.

Methods

The Cancer Genome Atlas (TCGA) Breast Cancer

TCGA BRCA ER(+)
808 patients

Documented Endocrine therapy
455 patients

SERM treated
222 patients

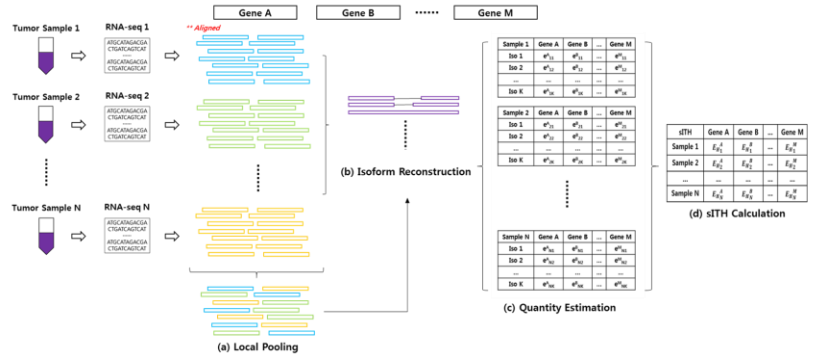
AI treated
295patients

Relapse (Resistant)
48 patients

No Relapse (Responsive)
407 patients

Differential Analysis of Splice Variants
in 94 *ESR1* Pathway-related Genes

Data-mining Algorithm for Recognizing Spliceomic Heterogeneity



Potential Spliceomic Signatures

Seoul National University Hospital Data

Whole Transcriptome Sequencing Data
120 patients

HR(+) and Endocrine Treated
59 patients

Relapse (Resistant)
24 patients

No Relapse (Responsive)
35 patients

Differential Analysis of Splice Variants
in Potential Spliceomic Signature Genes

Data-mining Algorithm for Recognizing Spliceomic Heterogeneity

Validation of Potential
Spliceomic Signatures

Spliceomic Signature

Results

Table 1. Potential Spliceomic Signatures From TCGA Data

Gene	Mean Isoform % in Resistant Specimens	Mean Isoform % in Responsive Specimens	p-value
AKT1	14.93	10.81	0.0033
ATF2	2.13	0.15	0.0038
ATF4	12.52	7.72	0.0044
CALM2	37.86	29.48	0.0451
CALM3	0.01	0.00	0.0403
CREB1	2.64	0.50	0.0028
EGFR	6.58	2.89	0.0008
ESR1	18.56	12.70	0.0050
ESR2	14.28	9.41	0.0243
GRM1	11.16	5.59	0.0209
HRAS	58.41	51.77	0.0041
HSP90AA1	4.93	1.29	0.0283
OPRM1	4.47	0.30	0.0160
PIK3R3	0.02	0.00	0.0093
PRKACB	0.47	1.02	0.0041
SHC1	46.30	49.13	0.0330
SHC4	0.02	0.00	< 0.0001

17 genes with dominant isoforms

- ✓ 419 Responsive patients (no relapse)
- ✓ 52 Resistant patients (relapse)

Results

Table 2. Potential Spliceomic Signatures Reproduced From SNUH Data

	Hormone Receptor Positive			Hormone Receptor Negative		
Gene	Mean Isoform % in Resistant Specimens	Mean Isoform % in Responsive Specimens	p-value	Mean Isoform % in Resistant Specimens	Mean Isoform % in Responsive Specimens	p-value
HRAS	64.47	57.14	**0.0037**	57.97	58.85	0.8413
SHC1	25.53	32.33	**0.0456**	28.36	32.58	0.2551
ESR1	31.83	27.54	0.4333	13.36	15.61	0.6466

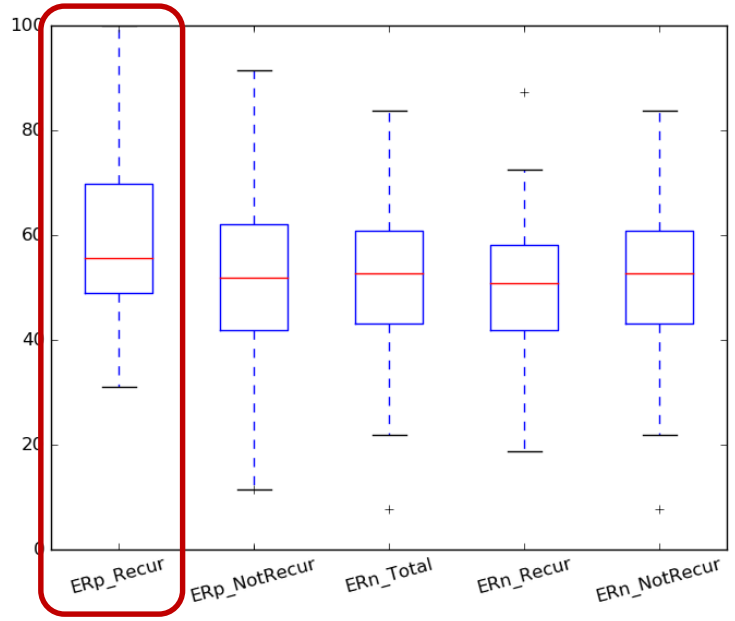
✓ 24 Resistant patients (relapse)

✓ 35 Responsive patients (no relapse)

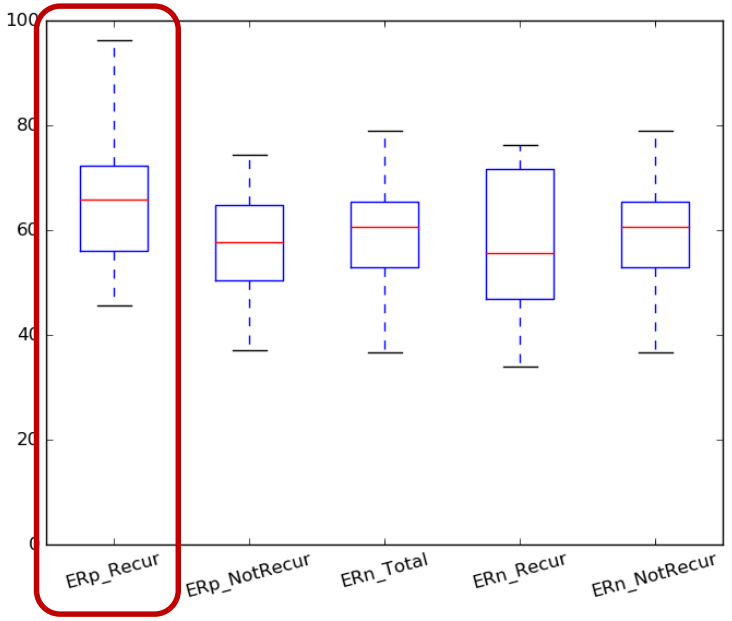
✓ 61 HR-negative (no relapse)

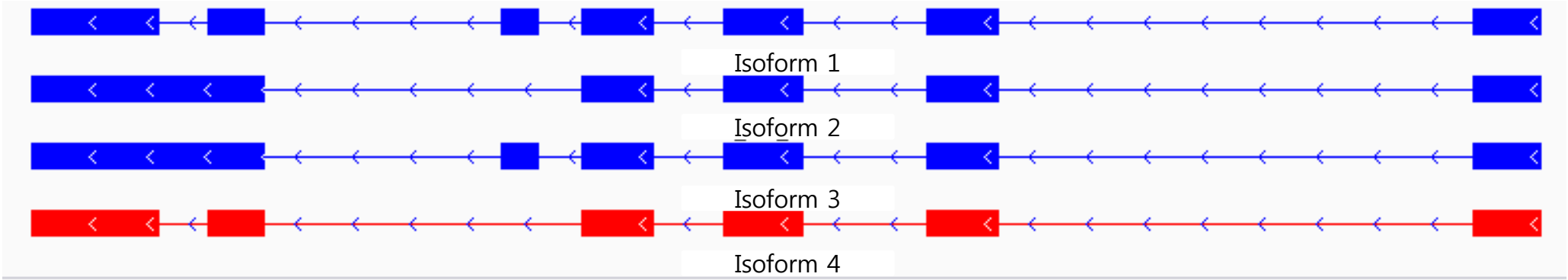
HRAS (Hras Proto-Oncogene, GTPase)

TCGA – HRAS signature isoform



SNUH – HRAS signature isoform

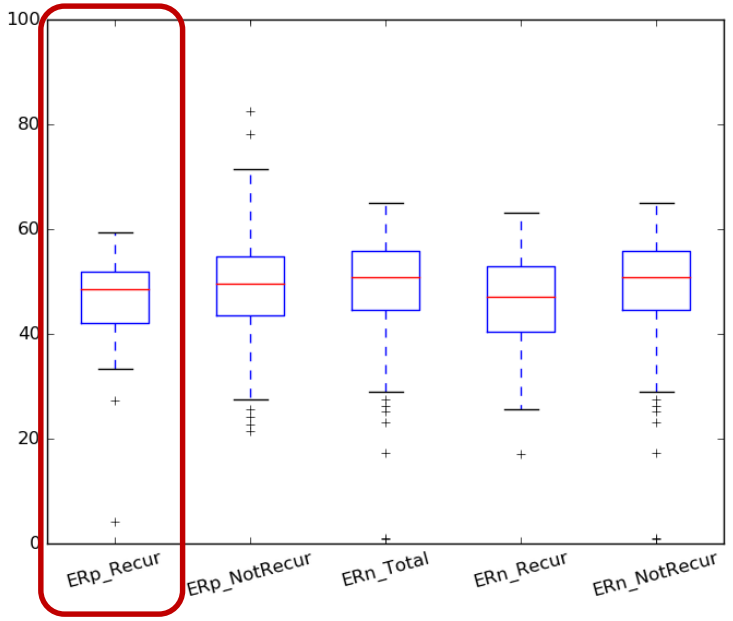




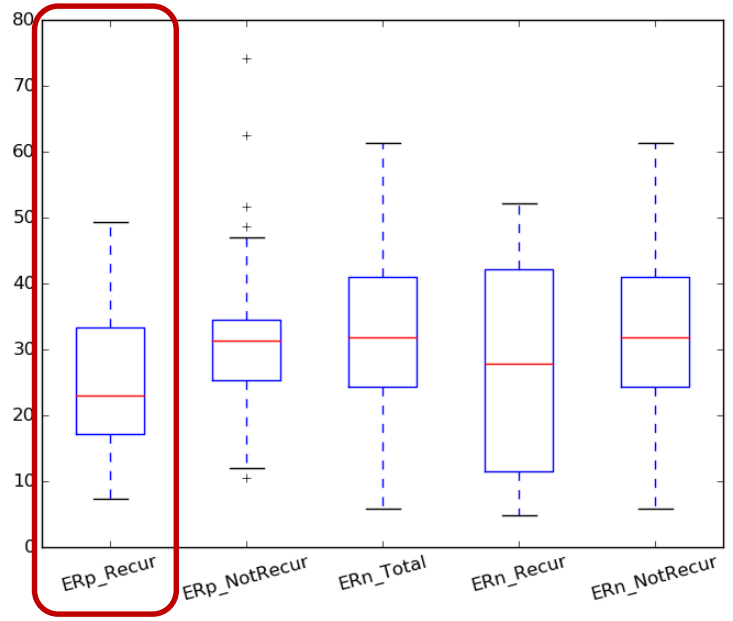
Isoform 4: 3'UTR exon splitting and 5th exon skipping

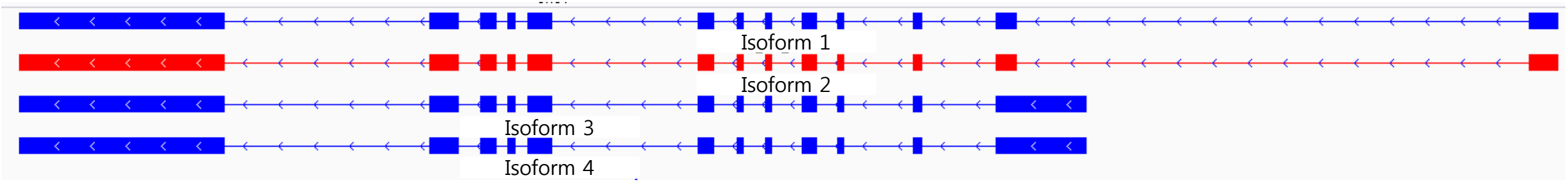
SHC1 (SHC (Src Homology 2 domain-containing) Adaptor Protein 1

TCGA – SHC1 signature isoform



SNUH – SHC1 signature isoform





Isoform 2: 5'UTR exon splitting and alternative 11th exon

Conclusions

- **Phenotype-specific splice variants** can be detected using transcriptome sequencing data.
- Splice variants in ***HRAS*** and ***SHC1*** are potential spliceomic signatures that may be used to predict endocrine therapy-resistant breast cancer.
- Further investigation is warranted to explore the biological role of these isoforms and identify the role of splice variants as a biomarker for endocrine resistance.

Future Plans

Potential Spliceomic Signature

- ✓ Other subtypes
- ✓ Other Pathway-related genes
- ✓ Other cancer types

Functional Studies

- ✓ Isoform overexpressed or depressed cell lines
- ✓ Functional assays
- ✓ Drug sensitivity

Validation

- ✓ Targeted RNA-seq
- ✓ Large scale FFPE tissue with clinical data
- ✓ Prognostication

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Patients and Family

Thank you for your attention